THE BREEDING HABITS OF *AMBLYSTOMA PUNCTATUM* LINN [1]

BERTRAM G. SMITH

On April 9, 1906, in some small ponds in a wood in the vicinity of Ann Arbor, Michigan, Prof. Jacob Reighard discovered what he surmised to be spermatophores of Amblystoma. At his suggestion and under his direction, I undertook the identification and study of these structures.

I. Observations

A. **The Spermatophores.** The spermatophores look like bunches or tufts of snowy-white fungus growing on leaves, twigs, or stalks of grass lying on the bottom of the pond. They invariably occur on a horizontal surface, and are never attached to an erect twig or stalk as is often the case with the eggs of Amblystoma. They are found in water from 6 to 10 inches deep, and 5 to 10 feet from the shore. The spermatophores usually occur in groups of about 40 or 50, but the number is extremely variable, ranging from 1 to 100. Isolated spermatophores are rarely found, though a single one is conspicuous enough to be readily discovered. The spermatophores of each group are scattered over an area of rather more than one square foot. Along the shores of an elliptical pond about 125 feet in length, 25 groups of spermatophores were counted; they were less numerous in three other ponds examined.

The spermatophores (Fig. 1.) resemble those of *Triton* (*Diemyctylus*) *viridescens* as described by Jordan ('91 and '93) rather than the more complicated structures produced by some European forms (Zeller, '05). Each consists of a base and a stalk of clear gelatinous material almost invisible in the water, having the general form of the stump of a tree, this structure is surmounted by a slightly broader cap or tuft of snowy-white felt-like material consisting of spermatozoa with no visible matrix. The material con-

[1] Contributions from the Zoological Laboratory of the University of Michigan, No.

stituting the base must be strongly adhesive when fresh, for the spermatophore is firmly attached to the object on which it is deposited. The cap is usually hemispherical in form, with the convex surface upward; but the material of which it consists often runs down the side of the stalk, or is found projecting in downy tufts like the cotton from an open cotton-boll. In many cases the caps have a frayed appearance, as if they had been disturbed; in occasional specimens the cap of spermatozoa is partly or almost wholly absent. The appearance in the latter case is like that of a spermatophore of *Triton viridescens* from which I have seen the ball of spermatozoa taken up into the cloaca of a female. The dimensions of the complete spermatophore are about as follows:

Height....................	6–8 mm.
Breadth of base............	6–8 mm.
Diameter of stalk near top...	2.5–3 mm.
" " cap............	3–4 mm.

As compared with some spermatophores of *Triton viridescens* obtained from specimens in captivity, these under discussion are slightly taller, with a smaller base and a stalk of much larger diameter, surmounted by a larger mass of spermatozoa. The spermatophore of *Triton viridescens* has a broad flattened base from the center of which rises a distinctly conical stalk tapering to a very slender spine, at the top of which is attached a small ball of spermatozoa; the spermatophores attributed to Amblystoma are more massive and more nearly cylindrical.

When found on April 9 and 10 the spermatophores were all in good condition, with some slight differences in the freshness of their appearance. In two or three days they became infested with fungus, disintegrated quite rapidly, and in a week very few few of them could be found. Had new ones been deposited in the interval, they could readily have been distinguished from the old ones; but no more spermatophores were deposited. Hence it is scarcely possible that the period during which spermatophores are deposited lasts longer than two or three days.

The spermatophores shown in the figure had been attacked by fungus and were beginning to disintegrate when photographed. The base is therefore no longer clear, but on the contrary the whole spermatophore appears white.

Identification. In order to identify the spermatophores, search was made for the parent animals. This resulted in the capture on April 11, of three specimens of *Amblystoma punctatum* Linn. which were found embedded in rotten wood under a stump at the edge of the water of one of the ponds where the spermatophores were numerous. From two of these specimens a few drops of seminal fluid, containing an abundance of spermatozoa, were obtained by stripping; from the third, which proved to be a female,

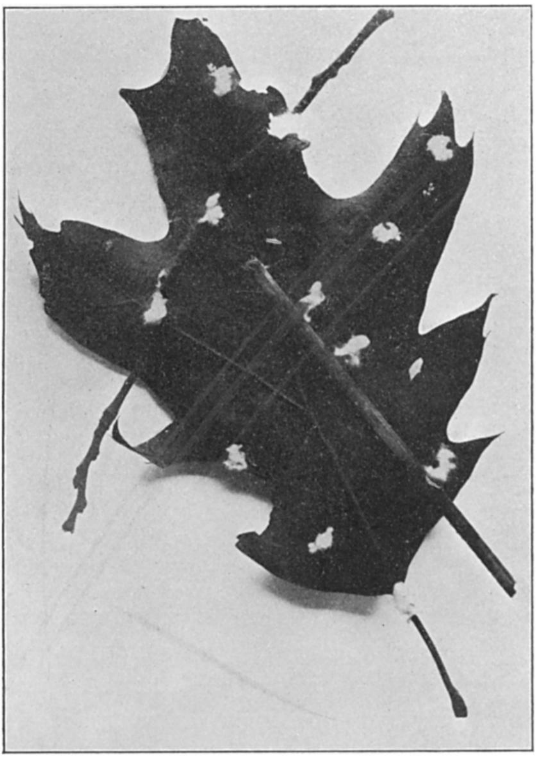

FIG. 1.— Spermatophores of *Amblystoma punctatum*. Two-thirds natural size, linear reduction.

comparatively few spermatozoa were obtained. The spermatozoa were mounted, stained, and compared with some taken from spermatophores and similarly treated. In structure, size, and staining reactions the two were identical.

Another species, *A. tigrinum* Green, also occurs in the vicinity

of Ann Arbor, and a single example was taken on April 9, in a field several hundred yards distant from the nearest pond where spermatophores were found; but the eggs of the two species are easily distinguishable, and in the case of *A. punctatum* were identified by means of eggs laid in the laboratory. With the exception of one bunch of eggs of *A. tigrinum,* all the eggs found in the pond where spermatophores were observed, were those of *A. punctatum.* With the single exception above noted, the two species have not been known to breed in the same ponds in the vicinity of Ann Arbor.

B. **The Spermatozoa.** The spermatozoon of *Amblystoma punctatum* is extremely long and slender. The head stains well with Delafields' haemotoxylin, the middle-piece less deeply. The tail-piece is bordered on one side by a very delicate undulating membrane. Some of the dimensions are as follows:

Length of acrosome	20 μ
" " head	106 μ
" " middle-piece	14 μ
" " tail-piece	480 μ
Total length	620 μ

The spermatozoon resembles in size and form that of *Triton viridescens,* with which it was compared, but the latter has a middle-piece twice as long, and a more conspicuous undulating membrane.

As compared with the spermatozoon of *Crytobranchus alleghe-niensis* (Smith '06) the sperm of *Amblystoma punctatum* is nearly three times as long, with a proportionally much longer middle-piece; the entire structure is much more slender and thread-like.

In freshly mounted seminal fluid the spermatozoa were seen in active motion. They tend to cling together parallel to each other to form bundles or ringlets, revolving with a circular motion; when so clustered they retain their vitality much longer than when separated. In a dying spermatozoon, long after the shaft has ceased to move, the activity of the undulating membrane continues. It gradually becomes slower until with a high magnification it is possible to follow a trough or a crest without interruption or change of form across the entire field of the microscope. The undulating membrane does not wind about the shaft as in Cryptobranchus, but continues on one side of it. When dead, the sperms are usually

found much convoluted, indicating a greater degree of flexibility than is the case with stouter spermatozoa like those of Cryptobranchus.

Experiments were performed to determine the length of time the spermatophores would retain their vitality in water, hence the interval within which they would have to be taken up by the female. In all the spermatophores examined the spermatozoa were motionless; but since the examination was not made until the evening of April 10, probably the spermatophores had been in the water for many hours. The effect of the cloacal secretion of the living female was then tried, to see if it would revive these spermatozoa; no such result was produced. Freshly obtained seminal fluid mounted in water retained its vitality for many hours; but as this experiment was not performed until April 18, only a small amount of seminal fluid could be obtained, and in this the sperms were not in a vigorous condition. If fresh seminal fluid were taken in the proper season and mounted in quantities to correspond with that deposited in a spermatophore, it might retain its vitality much longer. The viscous liquid in which the spermatozoa occur does not readily mix with water.

A freshly deposited spermatophore of *Triton viridescens* was obtained and kept in water; from time to time small portions of the ball of spermatozoa were teased apart and examined under the microscope. Eleven hours from the time the spermatophore was deposited, many active spermatozoa were found; an hour later all were motionless. Probably in an undisturbed spermatophore their vitality would be retained longer than twelve hours.

C. **The Eggs.** Those of *A. punctatum* have been described and figured by Clark ('80). The eggs, with their individual gelatinous envelopes, occur in compact bunches, surrounded by a very thick jelly mass. The entire structure is usually of an oval shape, often nearly as large as one's fist. The eggs of *A. tigrinum* are more loosely aggregated in a thinner jelly mass, and the cluster resembles a bunch of grapes. The clusters of eggs of *A. punctatum* are as a rule larger than those of *A. tigrinum*, and the number of eggs in a bunch is usually greater.

At the time of the discovery of the spermatophores, very few bunches of eggs could be found. The number steadily increased for a week; at the end of that time eggs were found in early seg-

mentation stages, showing that they had been quite recently laid. The egg-laying season follows immediately after the deposition of spermatophores, and lasts six or seven days. Nearly every bunch of eggs found on April 10 was close to a group of spermatophores.

On April 16, in the pond where 25 groups of spermatophores had been counted nearly a week before, about 55 bunches of eggs were found. Of these, many bunches were deposited in groups of two to four, probably by the same female. The number of aggregations of eggs very nearly equalled the number of groups of spermatophores.

D. **The Adults.** *Secondary Sexual Characteristics.* During the breeding season, at least, the cloacal region of the male is quite prominent; that of the single female examined was much less swollen, and the orifice was smaller. The cloaca of the male is lined with fine parallel papillated ridges, extending inward for a few millimeters; between these ridges are deep grooves, lined with cilia whose beat is outward. These ridges and grooves were not found in the single female examined. According to Kingsbury ('95) the female Amblystoma, as well as the male, has cilia in the cloaca but the tract is less extensive. The urogenital sinus of the male is larger than that of the female, probably to hold a considerable supply of seminal fluid preliminary to the deposition of a spermatophore. No secondary sexual characters to indicate the clasping of the female by the male were found.

II. Discussion.

Andrews ('97) described the structure and distribution of some spermatophores which he attributed to *Amblystoma punctatum,* but without positive identification. He states that these spermatophores were more slender and higher than those of *Triton viridescens,* and were distributed, at intervals of a few inches, in lines of several to a dozen. I find it difficult to reconcile his account with my own observations.[1]

[1] Professor Andrews, to whom the manuscript of this paper was submitted, writes,—"The spermatophores vary in size, arrangement and form here (about Baltimore) in different years and ponds; and I think your comparison with a tree stump a good one to indicate their common form. I judge the discrepancy in our account to be one of words rather than of observations. I am convinced from your photograph and account that you have described the same spermatophores that I did, and I judge both observations — despite some differences in descriptions — refer to Amblystoma punctatum."

On account of the late season at which my investigation was begun, no direct observations of the process of fertilization were possible. Clark ('79) says of some specimens of *A. punctatum* in confinement: "The males showed no inclination to clasp the females, but quietly deposited quite large masses of an apparently rather thick liquid, opaque white, on the bottom of the dish in which they were kept. Upon examination this was found to consist of spermatozoa moving actively in a liquid." The manner in which the spermatozoa reached the eggs was not observed.

Fertilization is undoubtedly internal. Of this the evidence adduced by Kingsbury ('95), and the presence of spermatozoa in the cloaca of the female as described above, furnish sufficient proof. It remains to consider how the transfer of spermatozoa is effected by the spermatophores.

The number of spermatophores is evidently very much greater than the number of females; and unless there exists an enormous disproportion between the sexes, each male must deposit a large number of spermatophores. Their abundance and the manner of their distribution, render it a very easy matter for the female to find enough of them for purposes of fertilization. In some portions of the pond it would seem scarcely possible for a female to move about in the water for any length of time without brushing against some of these spermatophores; hence there is the possibility of finding them by chance contact.

In the cases of those Urodela in which, as in *Triton viridescens* (Jordan '91 and '93; Hilton '02) and Axolotl (Gasco '81) the number of spermatophores deposited by a single male is small, particular safeguards are needed in order to facilitate their delivery to the cloaca of the female. In these forms the physiological necessity which requires the co-operation of the female in order that spermatophores may be deposited insures the presence of the female at the right time; subsequent reactions safeguard the reception of at least one of these spermatophores by the female cloaca. In *Triton viridescens*, according to my own observations, in some cases the female seems to make a definite attempt to get the spermatophore. The complicated behavior of the adults in these cases finds its biological significance not only in the increasing certainty of the process, but in a corresponding economy in the

number of spermatophores that must be deposited. With *Amblystoma punctatum*, on account of the very large number of spermatophores, there is the probability of a simpler mode of behavior, and the spermatophores may be found largely by chance. The wastefulness of the method is obvious. In Amblystoma as in Axolotl there is evidently no clasping of the female by the male, such as occurs in Triton.

The result of the experimental work on the vitality of the seminal fluid in water indicates that the spermatophore is not necessarily taken up by the female immediately after it is deposited; probably it is capable of effecting fertilization after exposure to the water for many hours.

On account of the shortness of the breeding season, the spermatozoa can be retained in the cloaca of the female for only a few days at most, before fertilization is effected. The position with respect to the spermatophores, of the earlier eggs found, suggests that in some cases the eggs are deposited immediately after the spermatophores are picked up.

The extreme flexibility of the sperm is doubtless correlated with the process of internal fertilization. In Cryptobranchus, in which fertilization is external (Smith '07), the egg envelopes must be penetrated after a brief exposure to the hardening effect of the water, and a much more rigid spermatozoon is required.

In the evolution of terrestrial from aquatic vertebrate life, a transition from external to internal fertilization takes place. External fertilization is not adapted to terrestrial conditions, hence in the land-living vertebrates it occurs only in some of the forms that revert to the water during the breeding season — *i. e.* in the Amphibia. Internal fertilization is an adaptation to terrestrial life in the sense that it is a condition antecedent to that life, not a result brought about by it; it may occur in purely aquatic vertebrates, as in the Elasmobranchs and a few Teleosts. Internal fertilization by means of spermatophores is a method still adapted to aquatic rather than to terrestrial conditions. It is a method intermediate between external fertilization on the one hand and internal fertilization without spermatophores on the other. Viewed in the light of the habits of the higher vertebrates, the occurrence of any method of internal fertilization in a form that breeds in the

water represents an advance upon the habit of external fertilization, and a stage in the evolution of habits that are to make possible the invasion and permanent occupation of the land.

Internal fertilization also finds a biological significance in the fact that in the course of its development there is gradually effected an economy in the amount of seminal fluid required for fertilization. This factor may account for the persistent development of the habit under aquatic conditions, where external fertilization is still possible; the incidental result is a preparation for terrestrial life.

In existing Amphibia we may find illustrations of various stages in this evolution of the breeding habits correlated with a transition from the water to the land. In Cryptobranchus, one of the lowest of the Urodela, leading an aquatic life and showing only in its methods of respiration and locomotion an advance toward terrestrial conditions, external fertilization takes place. This is evidently the primitive condition for the Urodela. In Amblystoma, a urodele living partly upon the land but returning to the water to breed, we see developed the peculiar habit of fertilization by means of spermatophores — a mode of internal fertilization favored by aquatic conditions. In *Triton viridescens* an economy of seminal fluid through a reduction in the number of spermatophores is made possible by definite reactions on the part of the adults, which insure fertilization. In the urodeles *Megapterna montana* Savi., *Molge aspera* Dugès and *Glossoliga Hagenmulleri* Lataste, according to Bedriaga ('82 and '95) the male emits spermatophores while still clasping the female; in *Triton torosus* Esch. (Ritter '99) it is probable that a very similar process occurs; in none of these cases, with the possible exception of *Molge aspera*, is there direct cloacal contact. Finally in the Apoda (the Sarasins '87–'93; Brauer '97) we find the establishment of a method of internal fertilization by direct cloacal contact, thus fulfilling the requirements for continuous residence upon the land.

University of Michigan Zoological Laboratory
Ann Arbor, Michigan

LITERATURE CITED.

Andrews, E. A.

1897. Breeding Habits of the Spotted Salamander (Amblystoma punctatum). Amer. Nat., Vol. 31, pp. 635–7.

de Bedriaga, J.

1882. Ueber die Begattung bei einigen geschwänzten Amphibien. Zool. Anz., Bd. V, pp. 265–268, 357–359.

1895. On the Pyrenean Newt, Molge aspera Dugès. Proc. Zool. Soc. 1895, p. 150.

Brauer, August.

1897. Beiträge zur Kenntniss der Entwicklungsgeschichte und der Anatomie der Gymnophionen. Zool. Jahrb. Anat., X, p. 389.

Clarke, S. F.

1880. Development of Amblystoma punctatum. Studies from Biol. Lab. of Johns Hopkins Univ., No. II.

Gasco, P.

1881. Les Amours des Axolotls. Zool. Anz., IV, pp. 313, 328.

Hilton, William A.

1902. A Structural Feature Connected with the Mating of Diemyctylus viridescens. Amer. Nat., Vol. 36, pp. 643–51.

Jordan, Edwin O.

1891. The Spermatophores of Diemyctylus. Journ. Morphol., Vol. X, No. 2.

1893. The Habits and Development of the Newt (Diemyctylus viridescens) Journ. Morphol., Vol. VIII, No. 2.

Kingsbury, B. F.

1895. The Spermathecæ and Methods of Fertilization in Some American Newts and Salamanders. Trans. Am. Micr. Soc., Vol. 17, pp. 261–304.

Ritter, W. E.

1897. Diemyctylus torosus Esch. The Life History and Habits of the Pacific Coast Newt. Proc. Cal. Acad. Sc., Third Series, Zoology, Vol. I, No. 2, 1897.

Sarasin, P. B. and C. F.

1887–1893. Zur Entwicklungsgeschichte und Anatomie der Ceylonischen Blindwühlen, Ichthyophis glutinosus. Ergebnisse naturwissenschaftlicher Forschungen auf Ceylon, II.

Smith, Bertram G.

1906. Preliminary Report on the Embryology of Cryptobranchus allegheniensis. Biol. Bull., Aug. 1906.

1907. The Life History and Habits of Cryptobranchus allegheniensis. Biol. Bull. (In press.)

Zeller, E. v.

1905. Untersuchungen über die Samenträger und die Kloakenwulst der Tritonen. Zeitschr. wiss. Zool., Bd. 79.